***ASJ**: International Journal of Health, Safety and Environment (IJHSE)*
Vol. 6 (09) 30 December, 2020, Pp. 711 – 720
www.academiascholarlyjournal.org/ijhse/index_ijhse.htm
ISSN: 2360-9311©Academia Scholarly Journals
Also Available@: Archive.org/Payal_et_al.
Open access

Full Length Research

Anti-spermatogenic Effects of Methanolic Extract of *Citrullus colocynthis* and *Delonix regia* on Male Reproductive Organs of Wistar Rats

Payal Soan[1], Ajit Kumar Sharma[1] and Ravi Sharma[2]*

[1]Department of Botany, St. Wilfred College for Girls, Mansarovar, Jaipur (Rajasthan), India.

[2]Department of Botany, K.R. College, Mathura; Ex-Founder Principal ESS ESS College of Education Dayalbagh, Agra and Retd. Prof. Botany Agra College, Agra (Dr. B. R. Ambedkar University Agra, Formerly Agra University, Agra) UP India.

*Corresponding Authors' Contact Details: E-mail Address ✉: drravisharma327@yahoo.com; Phone no ☎: + 91 9897258005

Accepted December 18, 2020

The present investigation was carried out in the laboratory Departments of Botany and Zoology, St. Wilfred College for Girls, Mansarovar and Reproductive Physiology and Endocrinology Section, Centre of Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India with fruit extracts of *Citrullus colocynthis* and *Delonix regia* on Male Reproductive Organs of Wistar Rats during (2018-2020) at Jaipur, India for evaluation of some andrological parameters such as morphology of spermatozoa, sperm count, motility, fertility index. The experiments were performed with fruit extracts (of *C. colocynthis* and *D. regia*) in double distilled water (100 mg/ml) administered orally to Wistar Rats randomly (RBD) divided into three groups with three replicates each: Group_1: Control Distilled water treated Rats; Group_2: Rats treated at 100 mg/kg of *C. colocynthis* extract 60 days and Group_3: Rats treated at 100 mg/kg of *D. regia* extract 60 days. Histopathological studies were carried out using the standard technique of double haematoxylin and eosin (HE) staining on male reproductive tissues (Testes) observed under binocular microscope taking microphotographs at different magnification with attached digital camera. It is found that as compared to control sets the plant extract treated rats showed degenerative changes in seminiferous tubules, decreased number of spermatogenic elements spermatozoa in testes reflecting anti-spermatogenic nature of the treatments: Initial body weight (g) V_1: Group_1 (Control) 134.00 > Group_2 (*C. colocynthis*) 130.50 > Group_3 (*D. regia*) 123.50; Final body weight (g) V_2: Group_1 (Control) 166.50 > Group_2 (*C. colocynthis*) 155.00 > Group_3 (*D. regia*) 153.30; Weight of Testes (g) V_3: Group_1 (Control) 1.58 > Group_3 (*D. regia*) 1.46 > Group_2 (*C. colocynthis*) 1.43; Sperm Motility (Cauda epididymides %) V_4: Group_1 (Control) 68.00 > Group_2 (*C. colocynthis*) 62.10 > Group_3 (*D. regia*) 55.00; Sperm Density million/ml (Testes) V_5: Group_1 (Control) 61.00 > Group_3 (*D. regia*) 40.00 > Group_2 (*C. colocynthis*) 35.10 and Fertility Index (%) V_6: Group_1 (Control) 97.60 > Group_3 (*D. regia*) 87.70 > Group_2 (*C. colocynthis*) 77.70. Furthermore, decreased weight of testes, sperm motility, sperm density and fertility indices support the androgen deprivation effects of the fruit extracts in rats.

Keywords: *Citrullus colocynthis*, *Delonix regia*, Fertility, Testis, Wistar rats.

INTRODUCTION

Use of medicinal plants in traditional systems of medicine for human health care has been done safely because they cause no side effects. Over 80% of the global population relies on traditional medicines, much of which is based on plant remedies. Traditional Chinese medicine alone uses over 5,000 plant species, folkloric medicinal use in the Philippines, Bangladesh and in India are extensively being carried out by tribal people. In recent past research on medicinal plants has attracted a lot of attention globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of medicine in the treatment of human diseases (Dutta et al., 1998; Mali et al., 2002; Abbas et al., 2006; Olaleye and Rocha, 2007; Mishra et al., 2008; Sharma et al., 2018). The ethano-medical properties of *Citrullus colocynthis* and *Delonix regia* have been reported earlier by different workers. *C. colocynthis* L. belonging to family Cucurbitaceae is a perennial trailing herb, native to Asia and Africa but found in North India, UP, MP, Rajasthan, Gujarat and South India, commonly known as bitter apple (Singh et al., 1986), also known as bitter cucumber, desert gourd. Bitter fruits are used as purgative and the extract from fruit pulp is highly effective against bacteria (Singh et al., 1983). *D. regia* Rafin., belonging to family Fabaceae commonly known as Peacock-flower and native Gulmohar, is a tree with an umbrella-shaped crown, native to Madagascar; grown in gardens and avenues for ornamental purposes and for shade (Singh et al., 1983). It is a naturalized tree in India widely grown as avenue tree It has been used in the traditional medicine systems of several civilizations. The fruit extract is known to have medicinal properties showing antimicrobial and antifungal activities. Since these plants occur locally and used for the treatment of various diseases in India with the hope that may affect the fertility ability of the treated Wistar Rats methanolic extracts of these plants were administered orally at the dose level of 100 mg/kg body weight for 60 days to evaluate their observed effects on testes and reproductive functions.

TESTES

The testes are the main male reproductive organs secreting testosterone and produce sperms a process called spermatogenesis (Carballada and Esponda, 1992). Each testis is covered by the tunica vaginalis testis, tunica albuginea and tunica vasculosa layers. The Tunica vaginalis consists of inner visceral and outer parietal layers. Beneath the visceral layer of the tunica vaginalis is the tunica albuginea followed by tunica vasculosa (a plexus of blood vessels and connective tissue). Tunica albuginea is divided into many pyramidal compartments called lobuli testes. At the end, seminiferous tubules straighten to form tubuli recti (also known as tubuli seminiferi recti, tubulus rectus, or straight seminiferous tubules) are structures in the testicle connecting the convoluted region of the seminiferous tubules to the rete testis. Each testicular lobule of testis contains one to three highly coiled seminiferous tubules made up of a single layered germinal epithelium. Testes contain three types of cell populations (Tortora and Derrickson, 2014). Inside the semniferous tubules are lined by two types of Germ cells: Sertoli cells and between the spaces in seminiferous tubules interstitial cells called Leydig cells are present. Sertoli cell is a kind of sustentacular cell (supporting cell), the "nurse" cell of the testicles that is part of a seminiferous tubule and helps in the process of spermatogenesis, the production of sperms activated by follicle-stimulating hormone (FSH) secreted by the adenohypophysis and has FSH receptor on its membranes. On the other hand, Leydig cells are the testicular cells responsible for the biosynthesis and secretion of androgens, which are critical for the development of the reproductive tract and for reproductive function in the male.

SPERMATOGENESIS

Spermatogenesis is the process in which sperms are formed from spermatogonial stem cells through mitosis and meiosis in the seminiferous tubule of the testes. Diploid spermatogonium mitotically divides to produce two diploid primary spermatocytes which are then converted into two haploid secondary spermatocytes. These haploid secondary spermatocytes undergo meiosis II to produce four haploid spermatids. During maturation process (Spermatogenesis) each spermatid losses extra cellular material like cytoplasm and some cell organelles except for mitochondria, nucleus, and acrosome centriole etc. Maturation process takes

place under the influence of testosterone. Testosterone binds to androgen binding protein (a protein of sertoli cells) present in the seminiferous tubule and initiate maturation of sperms (Eberhard and Shkaratan, 2012). These spermatozoa are transported to the epididymis where they become active and gain motility.

MATERIALS AND METHODS

Pharmacological Activity (Antifertility Activity)

Identification of Plants

Two plants, *Citrullus colocynthis* L. (family Cucurbitaceae) and *Delonix regia* Rafin., (family Fabaceae) specimens were collected around Jaipur district, Rajasthan, India and authenticated from herbarium at the Department of Botany, University of Rajasthan, Jaipur (India) and voucher specimen were deposited.

Preparation of Crude Extract

Fruits of the two plant species *Citrullus colocynthis* and *Delonix regia* were washed, shade dried and dried in oven at 40°C before grinding into a fine powder using the blender. One hundred (100) g of powder of fruits of each plant was mixed with distilled water and Methanol (1:1 vol) in a beaker and kept in water bath at 55°C for 12×4 hrs. The extract was then filtered through muslin cloth and further using Whatman No. 1 chromatographic filter paper. The filtered extract was evaporated in rotary evaporator at 80°C and completely dried in oven at 4°C to obtain the powder of extract which was stored at 4°C till further use.

Animals

The present investigation was carried out on mature adult male Wistar rats (weighing between 100-150 kg/ms) procured from local animal suppliers, Jaipur and acclimatized before starting the experiment. They were housed in polypropylene cages in the animal house under standard conditions of humidity, temperature (25 ± 2°C) and light (12 hr. light/dark). They were fed with standard rat pellet diet obtained from Ashirwad Pvt. Ltd Chandigarh, India and water was provided *ad libitum*. The study was approved by Institutional Animal Ethical Committee, School of Basic and Applied Sciences, Department of Zoology, Poornima University, Jaipur, Rajasthan (India), approved the study (2014 PUSBAPHDO08403 dated 6th May, 2017).

Experimental Design

All animals were divided into three groups for treatment each containing ten (10) animals. A total of 30 male fertile healthy Wistar rats were randomly divided into three groups as follows:

Group_1: Control treated vehicles double Distilled water.

Group_2: Rats treated at 100 mg/kg of ***Citrullus colocynthis*** extract 60 days.

Group_3: Rats treated at 100 mg/kg of ***Delonix regia*** extract 60 days.

Required amount of drug (extracts) was prepared freshly in double distilled water (100 mg/ml) and administered orally daily at 100 mg/kg for 60 days. The drug dose level was calculated according to LD_{50} and its ranges were calculated by the method following Litchfield and Wilcoxon (1949).

Histopathological Studies

Histopathological studies were carried out using the standard technique of double Haematoxylin and eosin (HE) staining. Male reproductive tissues were dissected out, blotted free of blood and fixed immediately after the autopsy. Fixation was carried out at room temperature for 24 hrs. Cut into 0-6 mm thick pieces and thoroughly washed overnight under running tap water. These tissues were transferred to 70% alcohol giving several changes. Thereafter tissues were dehydrated in ethanol series, cleared in xylene, embedded in paraffin wax at 55°C and transverse section (TS) were cut at 5 µm in Rotary Microtome for staining.

The sections were de-paraffinized and hydrated through xylene, alcohol series and distilled water then immersed in haematoxylin. After 5 min sections were thoroughly washed under running tap water. The sections were rinsed in 70% ethanol counterstained with eosin, differentiated and dehydrated in alcohol series, cleared in xylene and mounted in DPX. All the stained slides were observed under binocular microscope and photographs were taken at different magnification with attached digital camera.

Parameters Studied

A known amount of cauda epididymis was teased gently in a definite volume of normal physiological saline to release the spermatozoa form the epididymal tubules. The tissue components were removed and sperm suspension used for evaluating sperm function parameters such as sperm count (sperm density) and sperm motility.

Body Weight

The initial and final body weights of the animals were recorded.

Sperm Motility

The sperm motility was measured by the method of Prasad et al., (1972).

Sperm Count/Density

Cauda epididymal sperm count of all control and treated groups of animals were determined by the method of Prasad et al., (1972) using Neubauer chamber of haemocytometer.

Fertility Index

Treated males were cohabitated with normal adult cycling females in the ratio of 1:2 form 55 day of treatment. Thereafter number of pregnant females was counted to get fertility for 5 successive day index. The fertility index of control and treated groups of animals were calculated by formula mentioned below:

$$\text{Male fertility index} = \frac{\text{Number of males impregnating female}}{\text{Number of males cohabitated}} \times 100$$

DATA ANALYSIS

The values were expressed as SEm ±. The significance of difference among the groups was assessed using students "t"- test (Steel and Torrie, 1996). Symbols represent statistical significance as indicated a P ≤ 0.05, b 0.01, c 0.001 and 'ns' for Non-significant.

P ≤ 0.05 → a
P ≤ 0.01 → b
P ≤ 0.001 → c

CALCULATIONS

Statistical Data calculations were based on the bio-statistics. Standard error of the mean calculated by following formula:

$$S.E.(\sigma_{\bar{x}}) = \frac{\sigma}{\sqrt{n}}$$

Where,
SD = Standard deviation
n = no. of set
The significant test was calculated by the formula as given-
Standard deviation
SD =

Mean = $\bar{x} = \frac{1}{N}\sum_{i=1}^{N} x_i$
Where,
The mean is often denoted as $\bar{\mathbf{x}}$, pronounced "x bar," and even in other uses when the variable is not **x**, the bar notation is a common indicator of some form of mean. In the specific case of the population mean, rather than using the variable $\bar{\mathbf{x}}$, the Greek symbol mu, or **μ**, is used.
Students "t" test (t) =

$$t = \frac{M_1 - M_2}{\sqrt{\frac{{SD_1}^2}{N_1} + \frac{{SD_2}^2}{N_2}}}$$

Degree of freedom (df) = $(n_1+n_2) - 2$
Where,

m_1 = mean vale of control
m_2= mean vale of treated
SD_1 = Standard deviation of first set of values
SD_2 = Standard deviation of second set of values
n_1 = Total number of values in first set
n_2 = Total number of values in second set.

RESULTS

Plant Fruit Extract Treatment Effects on Body Weight and Weight of Testes

Non-significant changes were observed in final body weight of Wistar Rats treated with plant fruit extracts of *C. colocynthis* and *D. regia* in comparison to control treated vehicles (Tables1-3) which is shown in decreasing order as:
Initial body weight (g) V_1: Group_1 (Control)

Table 1. Effect on body weight, weight of testes, sperm motility, sperm density and fertility index of Wistar rats treated with fruit extracts of *C. colocynthis* and *D. regia* at 100 g/kg for 60 days.

SN	Groups	Body weight (%)		Weight of Testes (mg/100 g) V_3	Sperm Motility (Cauda epididymides %) V_4	Sperm Density million/ml (Testes) V_5	Fertility Index (%) V_6
		Initial (g) V_1	Final (g) V_2				
1	Group_1 Control	134.00 ± 3.055	166.50 ± 3.337	1179.00 ± 2.745	68.00 ± 0.258	61.00 ± 0.471	97.60 ± 0.542
2	Group_2 *Citrullus colocynthis*	130.50 ± 3.001[a]	155.00 ± 2.773[a]	1094.50 ± 3.801[c]	62.10 ± 0.277[c]	35.10 ± 0.277[c]	77.70 ± 0.597[c]
3	Group_3 *Delonix regia*	123.50 ± 2.570[a]	153.30 ± 2.761[a]	1138.50 ± 2.668[c]	55.00 ± 0.715[c]	40.00 ± 0.715[c]	87.70 ± 0.597[c]

Data expressed as SEm ± and significance at P≤ 0.05 a, P≤ 0.01 b and P≤ 0.001 c

134.00 > Group_2 (*C. colocynthis*) 130.50 > Group_3 (*D. regia*) 123.50;
Final body weight (g) V_2: Group_1 (Control) 166.50 > Group_2 (*C. colocynthis*) 155.00 > Group_3 (*D. regia*) 153.30.

The weight of testes was decreased significantly in Wistar Rats treated with plant *C. colocynthis* and *D. regia fruit* extracts (Groups_2 and _3) treatment as compared to control Rats Group_1. (Tables1-3) which is shown in decreasing order as:
Weight of Testes (mg/100 g) V_3: Group_1 (Control) 1179.00 > Group_3 (*D. regia*) 1138.50 > Group_2 (*C. colocynthis*) 1094.50.

Changes in Sperm Motility in Rats with the Plant Fruit Extracts Treatment

The sperm motility was decreased significantly in rats treated with plant *C. colocynthis* and *D. regia* fruit extracts treatment in comparison with controls (Tables1-3) which is shown in decreasing order as:
Sperm Motility (Cauda epididymides %) V_4: Group_1 (Control) 68.00 > Group_2 (*C. colocynthis*) 62.10 > Group_3 (*D. regia*) 55.00.

Plant Fruit Extracts Treatment Effects on Sperm Count

The sperm density was decreased significantly in rats treated with plants *C. colocynthis* and *D. regia* extracts treatment in comparison to controls (Table-1-3) which is shown in decreasing order as:
Sperm Density million/ml (Testes) V_5: Group_1 (Control) 61.00 > Group_3 (*D. regia*) 40.00 > Group_2 (*C. colocynthis*) 35.10.

Effects on Fertility Index in Rats Treated with Plant Fruit Extracts

The fertility index was changed significantly in rats treated with plants *C. colocynthis* and *D. regia* extracts treatment with comparison with controls (Tables 1-3) which is shown in decreasing order as:
Fertility Index (%) V_6: Group_1 (Control) 97.60 > Group_3 (*D. regia*) 87.70 > Group_2 (*C. colocynthis*) 77.70.

Finally, the overall results for anti-spermatogenic effects of methanolic fruit extracts of *Citrullus colocynthis* and *Delonix regia* on male reproductive organs of Wistar Rats on percent over control basis in the three Groups show clear cut decrease in all the parameters studied in Group_2 and Group_3 Wistar rats treated with *C. colocynthis* and *D. regia* fruit extracts as compared with Control Group_1 (Table 4). Further, the effect was lesser in the treatment with *C. colocynthis* fruit extract on Final body weight V_2; Weight of testes V_3 and Sperm motility V_4 of the treated Wistar rats as compared with *D. regia* fruit extract treated rats (Table 4) on the other hand, treatment with *D. regia* fruit extract on Sperm density V_5 and Fertility index V_6 was observed to be lesser as compared to *C. colocynthis* treated rats (Table 4) as shown below in the three Groups in descending order:
Initial body weight (g) V_1: Group_1 (Control) 100% > Group_2 (*C. colocynthis*) 97.01% > Group_3 (*D.*

Table 2. Independent Samples "t" Test control and *Citrullus colocynthis*.

SN	Independent Samples Test							
1	**Variables**	**Group**	**Mean**	**SD**	**SEM ±**	**t value**	**Df**	**P value**
2	Body weight Initial (g) V_1	Control	134.00	9.661	3.055	0.817	18	0.424
		Citrullus colocynthis	130.50	9.490	3.001			
4	Body weight Final (g) V_2	Control	166.50	10.554	3.337	2.650	18	0.016
		Citrullus colocynthis	155.00	8.769	2.773			
5	Weight of Testes (mg/100g) V_3	Control	1179.00	8.679	2.745	18.022	18	0.000
		Citrullus colocynthis	1094.50	12.021	3.801			
6	Sperm Motility (Cauda epididymides %) V_4	Control	68.00	.816	.258	15.584	18	0.000
		Citrullus colocynthis	62.10	.876	.277			
7	Sperm Density million/ml (Testes) V_5	Control	61.00	1.491	.471	47.375	18	0.000
		Citrullus colocynthis	35.10	.876	.277			
8	Fertility Index (%) V_6	Control	97.60	1.713	.542	24.683	18	0.000
		Citrullus colocynthis	77.70	1.889	.597			

regia) 92.16%;

Final body weight (g) V_2: Group_1 (Control) 100% > Group_2 (*C. colocynthis*) 93.09% > Group_3 (*D. regia*) 92.07%.

Weight of Testes (mg/100 g): Gr 1 (Control) 100% > Gr 2 (*C. colocynthis*) 90.41%. > Gr 3 (*D. regia*) 89.00%

Sperm Motility (Cauda epididymides %) V_4: Group_1 (Control) 100% > Group_2 (*C. colocynthis*) 91.32% > Group_3 (*D. regia*) 80.88%.

Sperm Density million/ml (Testes) V_5: Group_1 (Control) 100% > Group_3 (*D. regia*) 65.57% > Group_2 (*C. colocynthis*) 57.54%.

Fertility Index (%) V_6: Group_1 (Control) 100% > Group_3 (*D. regia*) 89.86 > Group_2 (*C. colocynthis*) 79.61.

Histo-Pathological Changes in Testes of Wistar Rats Treated with Plant Fruit Extracts

The histo-pathological changes in testes of Wistar Rats treated with plants *C. colocynthis* and *D. regia* fruit extracts treatment with comparison to controls have been shown in the Histo-pathological Photomicrographs of testes showed degenerative changes in germinal epithelium, spermatocytes, spermatids and spermatozoa, number of sperms which decreased significantly after Haematoxylin and Eosin (HE) treatment (Figures 1-9).

DISCUSSION

Although many compounds have been used to control function of male reproductive systems especially testes to control fertility in male, however, herbal plants extracts have been also practiced in traditional system because they are safe. Since times immemorial many plants as either their extracts or metabolites have been used for fertility controls. Plants *Citrullus colocynthis* and *Delonix regia* (Nmila et al., 2000; Adam et al., 2001; Parekh and Chanda, 2007; Mehni et al., 2014) have been used traditionally to cure different diseases. Therefore, in present investigation methanolic fruit extracts of *C. colocynthis* and *D. regia* were prepared and administered orally in male Wistar Rats. The results of the study exhibited that treatment of the extracts in rats caused reduction in the weight of testes, sperm motility and sperm counts and degenerative changes in testes.

Since androgens, FSH and LH are essential for

Table 3. Independent Samples "t" Test control and *Delonix regia*

SN	Independent Samples Test							
1	Variables	Group	Mean	SD	SEM ±	t value	Df	P value
2	Body weight Initial (gm) V_1	Control	134.00	9.661	3.055	2.630	18	0.017
3		*Delonix regia*	123.50	8.127	2.570			
4	Body weight Final (gm) V_2	Control	166.50	10.554	3.337	3.047	18	0.007
5		*Delonix regia*	153.30	8.731	2.761			
6	Weight of Testes (mg/100g b.wt) V_3	Control	1179.00	8.679	2.745	10.581	18	0.000
7		*Delonix regia*	1138.50	8.436	2.668			
8	Sperm Motility (Cauda epididymides; %) V_4	Control	68.00	.816	.258	17.103	18	0.000
9		*Delonix regia*	55.00	2.261	.715			
10	Sperm Density million/ml (Testes) V_5	Control	61.00	1.491	.471	24.523	18	0.000
11		*Delonix regia*	40.00	2.261	.715			
12	Fertility Index (%) V_6	Control	97.60	1.713	.542	12.279	18	0.000
13		*Delonix regia*	87.70	1.889	.597			

Table 4: Effect on body weight, weight of testes, sperm motility, sperm density and fertility index of Wistar rats treated with fruit extracts of *C. colocynthis* and *D. regia* at 100 g/kg for 60 days (Data Expressed as percent over Control).

SN	Groups	Body weight (%)		Weight of Testes (mg/100 g) V_3	Sperm Motility (Cauda epididymides %) V_4	Sperm Density million/ml (Testes) V_5	Fertility Index (%) V_6
1		Initial (g) V_1	Final (g) V_2				
2	Group_1 Control	100%	100%	100%	100%	100%	100%
3	Group_2 *Citrullus colocynthis*	97.01%	93.09%	90.41%	91.32%	57.54%	79.61%
4	Group_3 *Delonix regia*	92.16%	92.07	89.00%	80.88%	65.57%	89.86%

Data expressed as SEm ± and significance at P≤ 0.05 a, P≤ 0.01 b and P≤ 0.001 c

the production of the normal sperm density, sperm motility the treatment caused degenerative changes in sperm to genesis. *C. colocynthis* and *D. regia* treatments inhibited spermatogenesis might be due to decreased level of male hormone testosterone since testosterone regulates the growth and development of reproductive organs and spermatogenesis (Sharma and Kalla, 1994). In the spermatogenesis process the Sertoli cells and Leydig cells co-operation is required for the development of seminiferous tubules and germinal cells. Histopathological observations of *C. c olocynthis* and *D. regia* fruit extracts treated rats showed reduction in the Leydig cells and degenerative changes evidenced that the treatment of extracts of these plants caused damage to spermatogenesis in the present study. Similar observations have been also reported by the previous researcher in the rat model (Raji and Bolarninwa, 1997). In the present study, increased androgen production after *C. colocynthis* and *D. regia* fruit extracts treatment is reflected by the

Figures 1-9: Showing Photomicrographs of Testes of Wistar Rats Treated with Fruit Extracts of Two Plants *C. colocynthis* and *D. regia*.

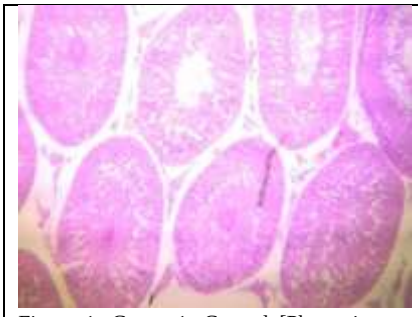

Figure 1: Group_1: Control [Photomicrograph of testis of rat showing normal feature 100X HE]

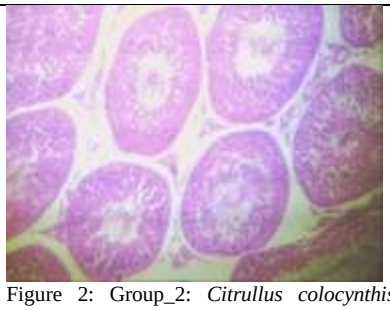

Figure 2: Group_2: *Citrullus colocynthis* treated [Photomicrograph of affected testis of rat showing reduced seminiferous tubules and cellular damage 100X HE]

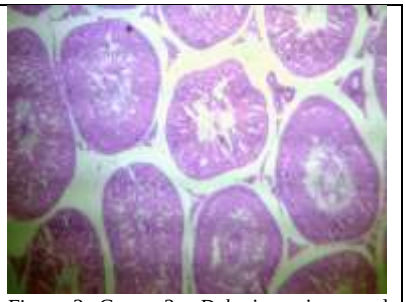

Figure 3: Group_3: *Delonix regia* treated [Photomicrograph of affected testis of rat showing reduced seminiferous tubules and cellular damage 100X HE]

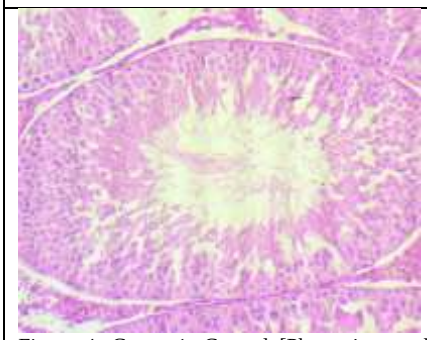

Figure 4: Group_1: Control [Photomicrograph of testis of rat showing normal feature magnified view 400XHE]

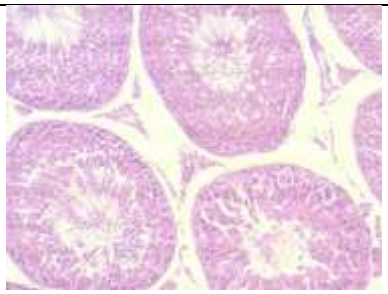

Figure 5: Group_2: *Citrullus colocynthis* treated [Photomicrograph of affected testis of rat showing reduced seminiferous tubules and cellular damage magnified view 400XHE]

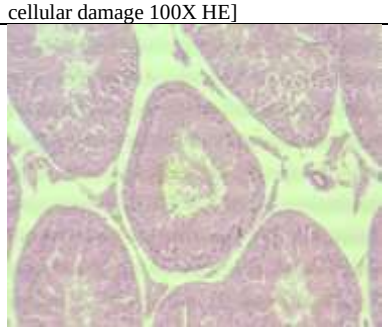

Figure 6: Group_3: *Delonix regia* treated [Photomicrograph of affected testis of showing reduced seminiferous tubules and cellular damage magnified view 400XHE]

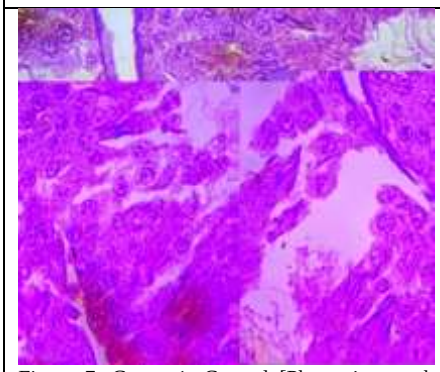

Figure 7: Group_1: Control [Photomicrograph of normal testis of rat under scanner]

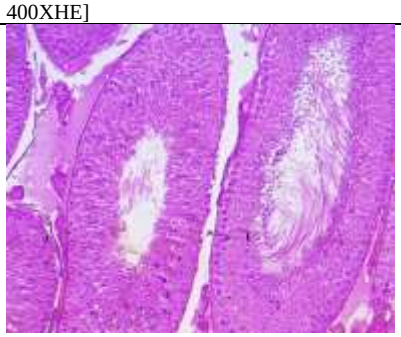

Figure 8: Group_2: *Citrullus colocynthis* treated [Photomicrograph of affected testis of rat showing reduced seminiferous tubules and CELLULAR damage under scanner]

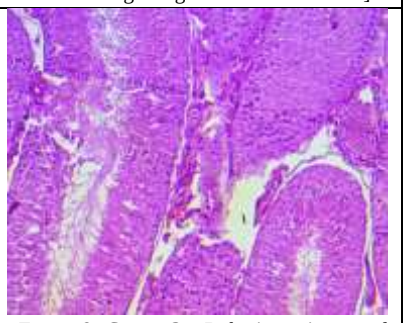

Figure 9: Group_3: *Delonix regia* treated [Photomicrograph of affected testis of rat showing reduced seminiferous tubules AND cellular damage under scanner]

increased number of mature Leydig cells and their functional status. It was also justified by the enhanced number of spermatocytes and spermatids as these stages are completely androgen-dependent (Robaire and Hermo, 1988). Methanolic extrats of *C. colocynthis* and *D. regia* treatment significantly reduced sperm density, sperm motility including fertility indices in fruit extract treated rats might be due decreased androgen levels.

CONCLUSION

Conclusively our results on Histopathological observations of extraction of both the plants *C. colocynthis* and *D. regia* on reproductive organs of model Wistar rats showed that there are significant degenerative appearance in seminiferous tubules as well as the number of spermatogenic elements also very low. Spermatozoa in testes had also

significantly reduced during higher dose of extracts of both the plants. Further, decline in weight of testes, sperm motility, sperm density and fertility indices support that of the *C. colocynthis* and *D. regia* fruit extract treatment, providing an evidence of the androgen deprivation effects of the extracts in Wistar Rats.

ACKNOWLEDGEMENTS

The authors wish to thank Principal St. Wilfred College for Girls, Mansarovar, Jaipur for kindly permitting and helping to work for the present investigation. Also, we thank HOD Department of Botany, University of Rajasthan, Jaipur for consulting herbarium and to HOD Reproductive Physiology and Endocrinology Section, Centre of Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India for work on Wistar rats and Photomicrographas.

REFERENCES

Abbas D, Simon G, Ali R, Hossein N, Masoud M, Lutfun N and Satyajtt D (2006). Flavone glycoside and cucurbitacin glycoside from Citrullus colocynthis. DARA.14(3): 109-114.

Adam M, Robert F, Larochelle M and Gaufreau L (2001). H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. Mol Cell Biol 21(18):6270-9.

Carballada R and Esponda P (1992). Maintenance or stimulation of steroidogenic enzymes and testosterone production in rat Leydig cell by continuous and pulsatile infusions of leutinizing hormone during passive immunization against gonadotropin- releasing hormone. J Reprod Fert. 95: 657-667.

Dutta BK, Rahman I, Das TK (1998). Antifungal activity of Indian plant extracts. Mycoses. 41(11–12): 535–536.

Eberhard A and Shkaratan M (2012). Powering Africa: Meeting the financing and reform challenge. Energy Policy. 42: 9–18.

Hama T, Shin KH and Handa N (1997). Spatial variability in the primary productivity in the East China Sea and its adjacent waters. Journal of Oceanography. 53 (1): 41-51.

Litchfiled JT and Wilcoxon FA (1949). Simplified method of evoluting dose effect experiments. J. Pharmacol. 96: 99-113.

Mali PC, Ansari AS and Chaturvedi M (2002). Antifertility effect of chronically administered Martynia annua root extract on male rats. Journal of Ethnopharmacology.82: 61-67.

Mehni AM, Ketabchi S, Hosein G and Bonjar S (2014). Antibacterial activity and polyphenolic content of Citrullus colocynthesis. International Journal of Biosciencs. 4(3): 190-196.

Mishra SB, Dwivedi S, Shashi A and Prajapati K (2008). Ethnomedicinal Uses of Some Plant Species by Ethnic and Rural Peoples of the Salem District of Tamilnadu with Special Reference to the Conservation of Vanishing Species. Ethnobot Leaflets. 12: 873-877.

Nmila R, Gross R, Rchid H, Roye M, Mauteghetti M, Petit P, Tijane RG and Sawaire Y (2000) Insulinotropic effect of Citrullus colocynthis fruit extracts. Planta Med. 66(5): 418-423.

Olaleye M and Rocha J (2007). Commonly used tropical medicinal plants exhibit distinct in vitro antioxidant activities against hepatotoxins in rat liver. Exp Toxicol Pathol. 58(6): 433-438.

Parekh J and Chanda SV (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J. Biol. 31: 53-58.

Prasad MR, Chinoy NJ and Kadam KM (1972). Changes in succinic dehydrogenase levels in the rat epididymis under normal and physiologic conditions. Fertil Steril., 23: 186-190.

Raji Y and Bolarninwa AF (1997). Antifertility activity of Cassia amara in male rats. Life Sci., 61: 1067-1074.

Robaire B and Hermo L (1988). Effect on ducts, epididymis and vas deferens structure, function and their regulation. In Knobil E Neil JD, eds., Physiology of Reproduction. New York, Raven Press Ltd, pp. 999- 1077.

Sharma M, Arya D, Bhagour K and Gupta RS (2018). Modulatory effects of methanolic fruit fraction of Pedalium murex on sulphasalazine-induced male reproductive disruption. Wiley andrologia doi. org: 10.1111/and.13190.

Sharma RK and Kalla NR (1994). Spermatozoal abnormalities and male infertility in the rat following sulfasalazine treatment. International Journal of Fertility and Menopausal Studies. 39: 347–354.

Singh U, Wadhwani AM and Johri BM (1986). Dictionary of Economic Plants in India. Indian Council of Agricultural Research (ICAR), New Delhi. Second Ed., Pp 51 and 68.

Steel RGD and Torrie JH (1996). Principles and Procedure of Statistics. A biometric approach (2nd ed.), McGraw-Hill, New York, pp. 6-15.

Tortora GJ and Derrickson BH (2014). Principles of Anatomy and Physiology by 14e with Lab Manual BIO 301 and 302 Purdue 3e Set. 16.